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Glyphosate pathways to modern diseases VI: Prions, amyloidoses and autoimmune neurological diseases

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Usage of the herbicide glyphosate on core crops in the USA has increased exponentially over the past two decades, in step with the exponential increase in autoimmune diseases including autism, multiple sclerosis, inflammatory bowel disease, type 1 diabetes, coeliac disease, neuromyelitis optica and many others. In this paper we explain how glyphosate, acting as a non-coding amino acid analogue of glycine, could erroneously be integrated with or incorporated into protein synthesis in place of glycine, producing a defective product that resists proteolysis. Whether produced by a microbe or present in a food source, such a peptide could lead to autoimmune disease through molecular mimicry. We discuss similarities in other naturally produced disease-causing amino acid analogues, such as the herbicide glufosinate and the insecticide L-canavanine, and provide multiple examples of glycine-containing short peptides linked to autoimmune disease, particularly with respect to multiple sclerosis. Most disturbing is the presence of glyphosate in many popular vaccines including the measles, mumps and rubella (MMR) vaccine, which we have verified here for the first time.

Contamination may come through bovine protein, bovine calf serum, bovine casein, egg protein and/or gelatin. Gelatin sourced from the skin and bones of pigs and cattle given glyphosate-contaminated feed contains the herbicide. Collagen, the principal component of gelatin, contains very high levels of glycine, as do the digestive enzymes: pepsin, trypsin and lipase. The live measles virus could produce glyphosate-containing haemagglutinin, which might induce an autoimmune attack on myelin basic protein, commonly observed in autism. Regulatory agencies urgently need to reconsider the risks associated with the indiscriminate use of glyphosate to control weeds.
Keywords: autism, autoimmune disease, collagen, glycine, glyphosate, multiple sclerosis, protein misfolding, vaccines

Assaults on health

One hundred and fifty years ago, in the heyday of Britain's industrial development and engineering works on a scale perhaps unparalleled in the history of mankind, there was little thought for the risks that might be associated with progress. In 1866, T.H. Huxley discoursed upon "all these great ships, these railways, these telegraphs, these factories, these printing presses, without which the whole fabric of modern English society would collapse into a mass of stagnant and starving pauperism" [1]. True, the Alkali Inspectorate had been formed in 1863, and sixty years earlier William Blake had written his famous poem referring to "dark satanic mills". But these were merely little clouds (to adapt a phrase of Lord Kelvin [2]) in a landscape of amazing hubris. For the last fifty years or so, we have become much more aware of the problems associated with material progress. It is as if, taking into account the turbulence brought about by two world wars, humanity needed time for the realities of the new order to sink in. A defining event was perhaps the appearance of the subsequently much-debated work by Forrester, Meadows and others reported as *The Limits to Growth* [3, 4]. And, ever-present in the background were the ideas of evolution brought to prominence above all by Darwin, and the problem of population, first effectively put on stage by Malthus [5, 4]. Another ingredient was Spengler's lucid explanation of the rôle of *die Maschine* [6, 7], perhaps less well known than it deserves to be in the English-speaking world, and epigrammatically summarized by Goethe's [8]

Wenn ich sechs Hengste zahlen kann, Sind ihre Kräfte nicht die meine? Ich

renne zu und bin ein rechter Mann, Als hätt ich vierundzwanzig Beine.

The machine is, essentially, a symbol of man's relentless and inextinguishable urge to dominate nature and is so deeply rooted, it must be considered ineradicable.

One of the most troubling clouds concerns the state of our health. The latest report on UK hospitals by the Care Quality Commission [9] paints a sombre picture of worsening health of the nation, despite having had seventy years of a comprehensive national health service. At the same time, the costs of health provision are constantly increasing as a proportion of gross domestic product (GDP) (which is itself increasing), not only in the UK but in most or all countries [10]. Admittedly, mean life expectancy is steadily rising but at the same time morbidity is expanding [11]. Part of the explanation is the continuing diminution of infant mortality and the fact that longevity implies a greater incidence of diseases specific to old age, but in between these two

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extremes we observe an alarming deterioration in the general health of populations.

Among the ten challenges promulgated by the United Nations' High-level Panel on Threats, Challenges and Change [12] are infectious diseases and environmental degradation. The former are felt to be more of a problem in the developing, rather than the developed, world, albeit that the ever-increasing ease of travel facilitates the spread of such diseases into the developed world. Nevertheless, the inexorable rise of microbes resistant to antibiotics is causing a resurgence of the dangers of endemic infectious diseases in the developed world [13]. This is very much a man-made situation. After antibiotics were discovered, there was a tendency to use them liberally and indiscriminately. The ideas of Darwinism were forgotten; it was perfectly natural that the microbes would evolve to circumvent the threats to their existence. The resulting problems have been greatly exacerbated by the use, in parallel with the strictly clinical applications, of antibiotics in animal husbandry [14], both as a way to compensate for laziness in the way animals are kept (their accommodation is not properly cleaned, encouraging disease, which is suppressed by the antibiotics) and because of an unexpected collateral effect of many antibiotics as growth promoters. In agriculture, a

similar laziness, this time manifesting itself as an aversion to digging and weeding, has encouraged the enormous (exponential) growth of glyphosate used as a weedkiller (Fig. 1), but in one of the glyphosate patents, antibiotic action is also claimed [15]. The sheer scale of glyphosate usage has rendered it ubiquitous in water and food [16], with doubtless far-reaching effects on microbial communities. Furthermore, because glyphosate is a total herbicide, it can be only used in agriculture in combination with food crops genetically modified to make them resistant to it, about which more anon.

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pigs and cattle given glyphosate-contaminated feed contains the herbicide. Collagen, the principal component of gelatin, contains very high levels of glycine, as do the digestive enzymes: pepsin, trypsin and lipase. The live measles virus could produce glyphosate-containing haemagglutinin, which might induce an autoimmune attack on myelin basic protein, commonly observed in autism. Regulatory agencies urgently need to reconsider the risks associated with the indiscriminate use of glyphosate to control weeds.

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1. INTRODUCTION

At first glance, multiple sclerosis (MS) and autism appear to have little in common, aside from the fact that both are neurological diseases. Autism is a condition with prenatal or early childhood onset, characterized by repetitive behaviours, impaired social interaction and cognitive impairment. The male:female ratio for autism is 4:1, while multiple sclerosis is twice as common in women as in men; its first symptoms usually begin in early adulthood to involve impaired lower limb mobility, although in later stages it affects both mental and physical capabilities. Both conditions are, however, associated with inflammatory autoimmune features [1, 2], and both diseases are viewed as having an environmental and a genetic component [3–6].

A study comparing a population of 658 MS patients with the general population found an association between MS and increased rates of asthma, inflammatory bowel disease (IBD), type 1 diabetes mellitus, pernicious anaemia and autoimmune thyroid disease [7], all of which

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have also been linked to autism [8–11]. These conditions are all considered to be *autoimmune diseases*, which can be triggered through molecular mimicry,

where an antibody responding to a foreign protein that resembles a native protein becomes sensitized to the native protein as well [12]. A paper by Shoenfeld and Aron-Maor in 2000 developed the argument that both autism and MS may be examples of an autoimmune reaction via mimicry following exposure to an antigenic stimulus, possibly from an infection or through vaccination [13]. They further propose specifically that myelin basic protein (MBP) and other proteins constituting the myelin sheath are attacked by the immune system in both autism and MS. This has been recognized by many others in autism [14, 15] and MS [16–20]. In 1982, Weizman et al. reported a cell-mediated autoimmune response to human MBP in 76% of the autistic children studied [16]. Immune sensitization to the myelin sheath proteins could arise either through mimicry as a consequence of exposure of the immune system to a foreign antigen with a similar peptide sequence that is

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resistant to clearance, or because the proteins themselves have been altered in some way that renders them defective, exposed and/or resistant to proteolysis.

Unlike DNA synthesis, protein synthesis is highly prone to error [21, 22]. It appears that biological systems have adopted a strategy of allowing coding errors to survive during active synthesis, but use protein misfolding as a criterion to mark a defective peptide for degradation and recycling through ubiquitination. It is estimated that 15% of average-length proteins will have at least one misincorporated amino acid. Typically, 10–15% of random substitutions disrupt protein function, mostly because of misfolding [22]. Such destabilization causes protein– protein aggregation, and can lead to multiple neurological diseases and amyloidoses. Drummond et al. propose that early-forming toxic oligomers of amyloidogenic proteins are enriched with missense errors [22].

Glyphosate is the active ingredient in the pervasive herbicide Roundup and in many other formulations of herbicides used to control weeds on agricultural, residential and public land worldwide. A recent study based in Germany

involving 399 urine samples from adults not involved in agricultural work revealed glyphosate residues above the detection limit in the urine of 32% of the subjects, and residues of AMPA, a metabolite, in 40% [23]. In a paper published in 2014, Swanson et al. showed a remarkable correlation between the rising rate of glyphosate usage on corn (maize) and soy crops in the USA and an alarming rise in a number of different chronic diseases [24]. Additional strong correlations for other conditions and diseases are provided in two follow-on papers [25, 26]. While correlation does not necessarily mean causation, causation becomes much more likely if a plausible mechanism can be found. Swanson et al. found a remarkable 0.98 correlation coefficient between the rise in autism rates in the USA and the use of glyphosate on crops (*P*-value δ $9.6 \cdot 10^{-6}$). The correlation for multiple sclerosis was not as high, but still highly significant at 0.83 (*P*-value δ $1.1 \cdot 10^{-5}$). IBD had a correlation coefficient of 0.94 (*P*-value δ $7.1 \cdot 10^{-8}$) (see Table 1 for other diseases).

Table 1. Correlations between time trends in several diseases and conditions recorded by the US Centers for Disease Control (CDC) with glyphosate usage on corn (maize) and soy crops reported by the USDA. Data reproduced from [23] and [25].

IBD, especially among children, is an emerging global epidemic [27] that is linked to autism [28, 29]. Impairment of intestinal barrier function is a core feature of IBD [30]. Increased intestinal permeability promotes infiltration of unmetabolized peptides into the lymph system and general circulation. This provides an opportunity for an immune antigenic response, which by molecular mimicry can lead to an attack on crucial proteins in the brain and spinal column. Disturbances of collagen texture are a major factor leading to the onset of diverticular disease and IBD along with the disturbed wound-healing mechanisms seen in the pathogenesis of anastomatic leakage following large bowel surgery [31].

In a recent paper [32], we suggested that glyphosate, a non-coding amino acid analogue of glycine, could substitute for glycine in error during protein synthesis. Such misincorporation and disruption of proteostasis could explain the strong correlations observed between glyphosate usage and multiple modern diseases. *In this paper, we show that this could be one of the most important mechanisms by which glyphosate could induce multiple autoimmune diseases*.

A prime site for initiation of the disease process is the colon, where misfolded collagen, resistant to degradation, could lead to an autoimmune disease and, subsequently, a leaky gut. Autoantibodies against type VII collagen have been detected in up to 68% of IBD patients [33]. Glycine is the most common amino acid in collagen, making up one fourth of the residues in the protein. Proline is also a very common component of collagen and, as we discuss later in this paper, proline resists hydrolysis. Incomplete collagen degradation by matrix metalloproteinases in the gut could lead to the accumulation of short pro–gly–pro peptides that are resistant to proteolysis. These could then induce the infiltration of neutrophils or the activation of resident immune cells to induce an inflammatory response [34].

An unpublished study conducted by Monsanto and submitted to the US Environmental Protection Agency (EPA) traced the accumulation of radiolabeled glyphosate in various tissues of rats following low-dose oral administration (10 mg/kg body weight) [35]. By far the highest accumulation was found in the bones (Table 11 in [36]). Radioactive levels in the colon were 4–6 times as high as those in the stomach and small intestine.

The production of novel non-coding amino acids by plants and microbes wards off predators. The toxicity of these products may be due to the fact that they replace coding analogues during protein synthesis. Examples include: azetidine-2-carboxylic acid (Aze), a proline analogue [37, 38]; glufosinate, a glutamate analogue that is also a popular herbicide [39]; ®-N-methylamino-L-alanine

Disease Autism (prevalence)

MS (deaths) IBD Anaemia Diabetes (prevalence) Thyroid cancer (incidence)

Correlation coefficient (R)

0. 98

0. 83 0. 94 0. 90 0. 97 0. 99

P-value 9.6×10^{-6}

1.1×10^{-5} 7.1×10^{-8} 1.8×10^{-4} 9.2×10^{-9} 7.6×10^{-9}

(BMAA), an analogue of serine [40]; and L-canavanine, a natural analogue of L-arginine that is exploited as an insecticide [41, 42].

A remarkable true-life story involving a 119-day Alaskan wilderness experiment conducted by Christopher McCandless was recounted in the book *Into the Wild* by Jon Krakauer (later made into a popular movie) [43]. McCandless was thought to have died in the wilderness from starvation; however, Krakauer always suspected a toxin in the seeds of the wild potato, *Hedysarum alpinum*, which formed a staple of his diet in his last month of life. Krakauer had originally suspected a poisonous alkaloid but, through later research, was able to identify a significant level of L-canavanine in the wild potato seeds and published a paper on this analysis with several other authors in 2016 [42].

A key factor in L-canavanine's toxicity is its ability to insinuate itself into peptides in place of L-arginine. L- canavanine can be assimilated into essentially any protein to create aberrant canavanyl proteins that can disrupt many fundamentally important biochemical reactions across a broad spectrum of organisms [41, 44]. L-canavanine is exploited in agriculture as a potent insecticide against the tobacco hornworm [45], although the tobacco budworm has developed tolerance with a unique enzyme, canavanine hydrolase, which can quickly metabolize it [46]. Larvae exposed to L-canavanine incorporate it into the protein lysozyme, resulting in a 48% loss in catalytic activity [41]. Furthermore, diptericins B and C of *Protoformia terranovae,* but not diptericin A, are negatively impacted by L-canavanine. The distinction is that diptericin A has histidine at position 38 instead of the L-arginine found in the other two diptericins. Presciently, with respect to glyphosate, Rosenthal wrote: "These insect studies support the view that the biological effects of canavanine result from its incorporation into a protein, resulting in an alteration in protein conformation that leads ultimately to impairment of protein function" [41].

2. SHIKIMATEPATHWAYINHIBITIONREVISITED

The shikimate pathway enzyme, 5-enolpyruvylshikimate- 3-phosphate

synthase (EPSPS) is believed to be the main target of glyphosate's toxicity to plants [47]. A 1991 paper by Padgette et al. describes studies to gain insight into the mechanism by which glyphosate disrupts EPSPS [47]. Surprisingly, it is not understood exactly how glyphosate binds to the active site.

The microbes *Klebsiella pneumoniae, Escherichia coli* [47, 48] and *Agrobacterium sp.* strain CP4 [48, 49] have all evolved to produce versions of EPSPS that are glyphosate-resistant. The CP4 variant has been widely exploited by importing it into genetically modified

glyphosate-resistant crops [48]. Insight can be gained by investigating the alterations to the peptide sequence that afforded resistance. All three mutations involved replacing a glycine residue at the active site with alanine [47, 48]. In the case of *E. coli*, the mutated enzyme is about 72 times *less* efficient than the wild-type enzyme, but 69 times *more* efficient in the presence of glyphosate. Changing the DNA code from glycine to alanine completely disables glyphosate's inhibiting effects on the enzyme [48].

Substitution of gly-96 at the active site in *E. coli* by serine leads to a version of the enzyme that is unable to bind PEP, most likely due to steric hindrance. The authors speculated that the hydroxymethyl group of serine displaces the phosphate of PEP and functions as a nucleophile. In fact, this mutated enzyme achieves a kind of reverse reaction, breaking EPSP down into shikimate- 3-phosphate and pyruvate via hydrolysis.

We propose that substitution of gly-96 (gly-100 in the CP4 variant) by glyphosate during protein synthesis could explain its disruption of the enzyme's function. One can expect that the highly reactive and bulky glyphosate molecule, if substituted for gly-96, would behave more like serine than alanine. An additional disruptive factor is glyphosate's chelation of manganese, which would disrupt the catalytic action of EPSPS. A cell containing both wild-type and glyphosate-substituted forms of the enzyme would arguably circuitously convert PEP to pyruvate via EPSP without producing ATP from ADP; i.e., would waste the energy in the phosphate bond, as shown in Fig. 1, and end up with excess pyruvate and a deficiency in EPSP.

Figure 1. Diagram of the hypothetical pathway by which glyphosate substitution for glycine in EPSPS could result in the synthesis of pyruvate from PEP without generating

ATP; i.e., wasting the energy in the phosphate group, as discussed in the text.

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3. GLYPHOSATEASAGLYCINEANALOGUE

While glyphosate's main mechanism of toxicity to plants is considered to be disruption of the shikimate pathway, it is also likely that it disrupts other biological pathways where glycine is either a substrate or a ligand, due to the fact that it is a glycine analogue. It has been proposed that, through glycine mimicry, glyphosate's rôle as a ligand to NMDA receptors in the brain could explain its known ability to activate NMDA receptors and cause neuronal damage [49, 50]. In [51], acute exposure of rat hippocampal slices to Roundup (0.00005–0.1%) for 30 minutes caused oxidative stress and neuronal cell death, which was attributed to NMDA receptor activation. Glyphosate also interferes with the synthesis of porphyrin, a precursor to haem, by disrupting the first step in the pathway where glycine is substrate [52].

N-substituted glycine "peptoids" are an attractive class of synthetic molecules that can be constructed by linking component N-substituted glycines at sequential nitrogen–carbon bonds; they are directly analogous to the linking of amino acids into peptides [53]. Glyphosate is of course an N-substituted glycine, where the nitrogen side chain is a methyl phosphonyl group. Part of the attraction of peptoids is that they are highly resistant to proteolysis, just as is the amino acid proline, in which the carbon side chain circles back and binds to the peptide nitrogen. Impaired ability to break down proline-rich gliadin has been proposed as a contributing factor in coeliac disease and gluten intolerance [54]. This can explain why common cereals with high proline contents are especially problematic to gluten-sensitive individuals [55, 56].

Glyphosate is probably particularly problematic when it substitutes for N-terminal glycines in proteins where these glycines are highly conserved and play a significant rôle. Several proteins rely on an N-terminal glycine for anchoring to the plasma membrane (e.g., endothelial nitric oxide synthase

(eNOS) [57]) or to the cytoskeleton (e.g., Kelch-like ECH-associated protein 1 (KEAP1) [58]). Protein N-myristoylation and prenylation depend on an amide bond to the N-terminal glycine residue [59]. For example, myristoylated G proteins involved in many signaling mechanisms depend on an N-terminal glycine residue [59]. This would be disrupted if the nitrogen atom has a side chain through glyphosate substitution for the terminal glycine.

N-nitrosoamino acids form a reasonable model for N-nitrosoglyphosate, a carcinogenic derivative of glyphosate that was of concern to the EPA during Monsanto's early studies. N-nitrosoproline is particularly relevant because proline, like glyphosate, has an extra carbon atom bound to the nitrogen atom. With respect to non-coding amino acids, and especially the incorporation

of N-nitrosoamino acids into peptides and proteins, R.C. Massey remarked: "In addition to their presence as free N-nitrosoamino acids, species such as N- nitrosoproline (NPRO) and N-nitroso-4-hydroxyproline (HONPRO) may exist in a peptide- or protein-bound form as a result of N-nitrosation of an N-terminal imino acid residue" [62]. Tricker et al. [63] and Kubacki et al. [64] devised high performance liquid chromatography– thermal energy analyser (HPLC–TEA) techniques for analysis of multiple dipeptides with a nitrosylated N- terminal, including N-nitrosoprolylalanine (NPROALA), N-nitrosoprolyl-4-hydroxyproline (NPROHOPRO) and N- nitrosoprolylglycine (NPROGLY) [63, 64]. Tricker notes that the average recoveries for NPROALA, NPROHOPRO and NPROGLY, 200 ⌈g of which was added to cured meat, were between 69 and 88%. Tricker also used the method to analyse the nitroso-tripeptide N-nitrosoprolylgly- cylglycine [65].

Nitrosamines of glyphosate (N-phosphonomethylgly- cine), its salts and esters include: N-nitrosoglyphosate (NNG) (Monsanto CP 76976), N-nitrosoiminodiacetic acid (NNIDA), N-nitrosoglyphosate sodium salt (NNGNa), N- nitrosoglyphosate isopropylamine ester (NNGIPA), N-nitrosoglyphosate potassium salt (NNGK), the metabolite N-nitrosoAMPA (NNAMPA), the metabolites N-nitrosodi- methyl amine (NDMA) and N-nitrosarcosine (NSAR), which occur in glyphosate products or may be generated *in vivo* or in soils and waterways. N-nitroso compounds derived from secondary amines are considered carcinogenic.

Monsanto glyphosate documents reveal analysis and quantification of five nitrosamines of concern [61]. Out of six lots of Roundup analysed for NNG, four lots contained NNG residues of 0.61 to 0.78 ppm and two lots had residues from 0.22 to 0.40 ppm NNG. Analysis of six lots of Monsanto Rodeo revealed NNG residues in the range 0.13–0.49 ppm.

Recently, a powerful metatranscriptome study on bacterial gene expression following glyphosate treatment was conducted on microbes growing within the rhizosphere of glyphosate-tolerant corn [66]. RNA transcript abundance was compared between control and glyphosate-treated samples in order to characterize which protein genes were upregulated or downregulated. While they found many changes in gene expression, most striking to us was the upregulation of genes involved in both protein synthesis and protein hydrolysis. The ribosomal proteins L16p (L10e) and Firmicutes ribosomal L7Ae family proteins involved in the synthesis of the ribosomal large subunit increased 1.4- and two-fold, respectively, and the small subunit ribosomal protein S11p (S14e) increased 1.5-fold. Upregulation of genes involved in protein degradation was even more dramatic. For

example, transcripts for a proteasome ® 2 subunit (EC 3.4.25.1) increased 4.3-fold and aminopeptidase YpdF increased threefold. An explanation could be an increase in the number of proteins that fail to fold properly due to glyphosate substitution for glycine in the protein. These authors also suggested a potential shift towards an increase in glyphosate-tolerant bacteria, a point that will become important later in this paper.

These results are corroborated by a study on pea plants grown in hydroponic culture, which revealed that glyphosate induced a significant increase in two major systems for proteolytic degradation: the ubiquitin-26 S proteasome system and papain-like cysteine proteases [67]. It also increased the total free amino acid content and decreased the soluble protein in the root system.

4. GLYPHOSATE-CONTAMINATED COLLAGEN AND PROTEOLYSIS RESISTANCE

We mentioned in the Introduction the gly–pro–gly peptide sequence that is common in collagen and linked to autoimmune disease. There are several enzymes in multiple organisms that are devoted to the proteolysis of peptide sequences containing proline, particularly the gly–pro sequence. These include enzymes that detach a terminal proline, enzymes that detach a dipeptide sequence where the second residue is a proline molecule and the first one is often glycine, and enzymes that break apart the X–pro dipeptide to release two free amino acids, one of which is proline. Certain pathogens have special modified versions of these enzymes, and there are genetic diseases related to pathologies in these enzymes. Substitution of glyphosate for glycine in this sequence is likely to cause extra stress to the enzymes that break down these sequences, potentially leading to autoimmune disease.

Prolyl aminopeptidase is an enzyme that detaches a terminal proline residue from a peptide. The enzyme is expressed predominantly by pathogenic bacteria in the gut, in particular *Serratia marcescens*, a common pathogen in the gut as well as in the urinary tract; it is often multiply antibiotic-resistant and is a serious threat in hospital-acquired infection [34]. This enzyme is especially important to the pathogens for degrading collagen, providing amino acids as fuel. It is conceivable that the pathogens are able to degrade glyphosate- contaminated peptides terminating in proline whereas the human form of the enzyme is not. It is intriguing that the *S. marcescens* version of prolyl aminopeptidase is unusual in having extra space at the active site [34], which could potentially accommodate the larger glyphosate molecule adjacent to the terminal proline residue. This might also contribute to glyphosate's observed effect on the gut microbiome: excessive growth of pathogens.

Multiple strains of the toxic mould *Aspergillus* secrete an X–prolyl dipeptidyl aminopeptidase (X-PDAP) that is important for digesting collagen because it can separate out an X–pro pair to bypass the difficult step of breaking the X–pro bond. Research has shown that this enzyme is essential for hydrolysing proline-containing peptides [69, 70]. It is likely that it becomes even more essential when X is glyphosate, as the peptoid sequence glyphosate–proline is likely almost impossible to break. Since gly–pro is a very common sequence in collagen, glyphosate–pro is likely to impede the breakdown of collagen fragments, which may then encourage *Aspergillus* infection in both plants and animals. Glyphosate has been shown to increase

the growth rate of *Aspergillus* [71].

The most disturbing question is, what happens in the absence of pathogens that can effectively clear collagen peptides contaminated with glyphosate? As we will see later in this paper, antibodies to collagen are linked to antibodies to vaccines. A genetic defect in the enzyme prolidase, which can break apart the very common gly–pro dipeptide to release the individual amino acids, leads to a severe disease with mental deficiencies and multiple skin lesions [72]. Intriguingly, a common plant pathogen, *Xanthomonas campestris*, which causes blight on multiple plant species has a unique variant of prolidase with two mutations, a substitution of tyrosine for gly-385 and valine for tyr-387, two highly conserved residues in the peptide sequence [73]. Is it possible that swapping out glycine affords protection from glyphosate substitution for this residue? We hypothesize that peptides derived from multiple proline and glyphosate-contaminated proteins, which are highly resistant to proteolysis, are causing an autoimmmune epidemic that is an important contributor to autism and other autoimmune disorders.

5. BMAAANDALSINGUAM

®-N-methylamino-L-alanine (BMAA) is another noncoding amino acid and an analogue of serine [40]. BMAA is synthesized by cyanobacteria, the microbes responsible for the toxic algal blooms that occur in lakes experiencing an accumulation of nitrogen and phosphate nutrients following hot, rainy weather [74]. An *in vitro* study by Dunlop et al. in 2013 demonstrated that BMAA can be misincorporated into human proteins, causing protein misfolding that could lead to neurological diseases [40].

BMAA has, in fact, been linked to several neurodegenerative diseases, including Parkinson’s, Alzheimer’s and amyotrophic lateral sclerosis (ALS) [75]. A 2013 study linked an ALS cluster in Chesapeake Bay to consumption of BMAA-contaminated crabs [76]. A study in France investigated an ALS cluster near a lagoon that supplied oysters and mussels to the local

population. The authors demonstrated that the shellfish were contaminated with BMAA, but also remarked that there was intensive chemical-based agriculture in the region [77]. Interestingly, cyanobacteria have been found to be remarkably resistant to glyphosate [78, 79], and this could contribute to the recent record-setting algal blooms in the Great Lakes region, where glyphosate is extensively used on genetically modified (GM) Roundup-Ready crops [80].

One likely molecule that could be adversely affected by BMAA is the glutamate transporter, whose defective expression has been linked to ALS [81]. Glutamate excitotoxicity in motor neurons is associated with ALS, and this could be caused by an impaired glutamate transport system. Ordinarily, astrocytes quickly clear glutamate from the synapse, following its release by neurons, and the transporter is essential for this clearance. A conserved serine-rich motif in the glutamate transporter forms a reëntrant loop, similar to a structure found in many ion channels [82]. This loop is crucial for the enzyme's proper function, and would be disrupted by substitution of BMAA for serine.

An interesting detective story has evolved around an epidemic of a complex neurological condition termed amyotrophic lateral sclerosis–Parkinsonism dementia complex (ALS–PDC), which reached epidemic proportions during a short interval after World War II among the native Chamorro people on the small island of Guam in the South Pacific. At the peak of the epidemic, the natives had a hundredfold increased risk to ALS and Parkinson's disease compared to the risk in the general human population.

A plausible explanation for this epidemic relates to a popular native food source: seeds from the cycad trees [83–85]. Cycad seeds contain BMAA, likely derived from associated cyanobacteria. However, what is especially interesting is that the BMAA becomes concentrated in the skin of fruit bats that feed on the cycad seeds. Fruit bats were a popular delicacy among the natives, who ate every part of them, including the skin. Increased access to firearms from the USA during the war may have made it easier to kill the bats, on which the natives then feasted, ultimately leading to the natives' near-extinction through the accumulation of BMAA in their brains [86]. Meanwhile the near-extermination of the bats through the hunting removed the presumed source of the epidemic [83].

However, the warfare also led to the accumulation of many toxic chemicals in the soil, which could have encouraged the proliferation of cyanobacteria, which are especially resilient in the face of stressors. The bats' demise was undoubtedly hastened by the accumulation of

excess BMAA in their tissues. A measurement of the amount of BMAA in three dried specimens of fruit bats from Guam taken from a museum in Berkeley found concentrations between 1200 and 7500 ⌈g/g, which indicates up to hundredfold bioamplification over the level in the seeds of the cycad tree [87].

There have been inconsistent results in measuring the levels of BMAA in different tissue samples, but this has been explained recently by the realization that any BMAA incorporated into proteins may be missed in analysis without sufficient proteolysis. Ince et al. wrote: "When the insoluble, protein-containing fraction following TCA (trichloroacetic acid) extraction is further hydrolysed to release BMAA from protein, there is a further pool of protein-bound BMAA that is present in a ratio of between 60:1 and 120:1 compared with the pool of free BMAA" [84, p. 348]. We believe that this point has great significance when it comes to glyphosate: we highly suspect that different methodologies used to measure glyphosate contamination in any situation where there is a significant protein-bound component may yield different results depending on the degree to which protein hydrolysis is carried out.

6. GLYPHOSATE CONTAMINATION IN COLLAGEN, ENZYMES, GELATIN AND V ACCINES

Gelatin is commonly used as an excipient stabilizer in vaccines, particularly the live virus vaccines. Gelatin is derived from animal skin and bone, especially of pigs and cattle; they may be fed glyphosate-contaminated forages, including GM Roundup-Ready corn and soy feed, which are sometimes supplemented with GM Roundup-Ready beet pulp. Gelatin is mainly derived by partial hydrolysis from the collagen in skin and bone. 26% of the amino acids in collagen are glycine; proline and hydroxyproline together make up 18% [88]; and glutamate constitutes 6%. All three of these components are problematic. The proline could be substituted by Aze from the sugar beet, the glycine could be substituted by residual glyphosate in the feed, and glutamate is a neurotransmitter but known to be neurotoxic at high

concentrations; it works together with glycine to excite NMDA receptors in the brain. The vaccine virus may incorporate some of the noncoding amino acids into its own proteins to produce versions of them that resist proteolysis and induce autoimmunity through molecular mimicry.

One of us (Samsel) analysed a number of animal protein products for glyphosate. These included the bones of pigs, cows, horses' hooves, bees and bee products, collagen and gelatin products, vitamins, protein powders, enzymes and vaccines. Results are shown in Tables 2 and 3. Both high performance liquid

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chromatography with tandem mass spectrometry (HPLC–MSMS) and enzyme-linked immunosorbent assay (ELISA) methods were utilized. It has been shown that both HPLC and ELISA are comparable in terms of accuracy and precision for detection and quantification of glyphosate in water-based analysis and including Nanopure, tap and river waters. Water-based solvents for

glyphosate demonstrate a detection limit of 0.6 ng/mL and a linear functional range of 1–25 ng/mL [200]. However, HPLC was not able to achieve detection below 5 ppb;[1] hence, in cases including water-based vaccines, analysis using numerous sample runs was made including using two independent labs to test the same samples.

Table 2. Residues of glyphosate found in animal-based products that were reported to the US Food and Drug Administration (FDA) by Samsel Environmental & Public Health Services. The limit of detection for glyphosate using hot water extraction is 0.075 parts per billion (ppb).[1]

Protein substrate

GELA TI N

GELA TI N

GELA TI N GELA TI N

ORAGEL

Type

JELL-O ORANGE #07 JAN 2018 DB02 02:36 POWER-MAX PROTEIN POWDER ADVANCED NUTRITION

DISNEY GUMMIES VITAMINS FLINTSTONES GUMMIES VITAMINS CHILDREN'S ORAGEL 7.5% BENZOCAINE FORMULA

Test date

Glyph osate residue (ppb)[1]

29 July 2016 9.00

29 July 2016 14.94

9 August 2016 8.27 9 August 2016 5.32

26 September 2016 2.81

HPLC–MSMS was also later used, where the method detection limit (MDL) permitted, for additional confirmation and quantification of glyphosate in digestive enzymes and collagens. Spiked sample recoveries were done for all samples tested. Freshly prepared glyphosate standard solutions were run as controls and results were calculated based on a standard curve.

In 1989, Monsanto researchers conducted an experi- ment on exposure of bluegill sunfish to ^{14}C-radiolabeled glyphosate [89]. One of us (Samsel) obtained the (unpublished) report from the EPA through the Freedom of Information Act. The researchers had found that, with EDTA extraction, the amount of radiolabel in tissue samples was much higher than the amount of detected glyphosate. They decided to apply a digestive enzyme, proteinase K, and discovered that this "caused a substantial improvement in extractability". It brought the yield from 17–20% in the case of EDTA to 57–70% following digestion with proteinase K. They summed up as follows: "Proteinase K hydrolyses proteins to amino acids and small oligopeptides, suggesting that a significant portion of the ^{14}C activity residing in the bluegill sunfish tissue was tightly associated with *or incorporated into* protein" (present authors' emphasis). In this context it is important to recall that a 60- to 120-fold higher detection level of BMAA was obtained following protein *hydrolysis* of contaminated proteins [84].

Since Monsanto found bioaccumulation of glyphosate in all animal tissues, with the highest levels in the bones and marrow [35, 36], one would expect that all tissues derived from animals fed a diet containing glyphosate residues and used for food by people around the globe would be contaminated. Knowing that the bioaccumulation of glyphosate would be evident in the vast majority of animals raised for market and fed a contaminated diet, as well as their products; and suspecting the possibility of contamination of even the digestive enzymes derived from these animals, one of us (Samsel) decided to analyse random samples.

Results from various gelatin-based products, along with the results for several different vaccines (discussed later) were reported to the FDA by Samsel Environmental & Public Health Services in August 2016. Table 2 shows results for glyphosate residues found in these gelatin-based products. The highest level found in a gelatin sample was almost 15 ppb.[1]

Having found glyphosate in animal gelatins, analysing the collagen at the source was a logical next step. Tissues from pork and cattle obtained from a local supermarket, commercially available collagen sourced from industrially-raised swine and oxen, as well as the purified digestive enzymes pepsin, lipase and trypsin, derived from pigs, were selected for evaluation. Three methods of laboratory analysis were used to determine if

[1] Parts per (US) billion. To put this into perspective, 1 ppb = 1 ⌠g/kg, and 1 ⌠g of glyphosate (N-phosphonomethylglycine) contains $3.561 \cdot 10^{12}$ molecules of the substance, each one of which could integrate with a protein.

glyphosate was present in porcine pepsin and in the glycine-rich collagen from the tissues of pigs and cattle, protein sources that are regularly consumed by Americans. The results are given in Table 3.

Glyphosate integration with enzymes is a serious consideration, as glyphosate may serve as an enzyme inhibitor like other phosphonates [90–92]. Inhibition and immobilization of enzymes may occur via three basic categories:

covalent linkage; adsorption on a carrier; or entrapment within macromolecules [93].

Inhibition of enzymes may be reversible or irreversible. Types of reversible enzyme inhibition include competitive, noncompetitive and uncompetitive. *Irreversible* inhibitors covalently bond to the functional groups of the active site, thus permanently inactivating catalytic activity. Irreversible inhibition includes two types: group-specific inhibition and “suicide” inhibition.

The importance of fully functional digestive enzymes cannot be understated. They are essential for metabolic function, as they convert food into nutrients and other molecules that are then available to cells for tissue and organ growth, maintenance and repair. The precursor trypsinogen, produced in the pancreas, is enzymatically transformed into the serine protease trypsin. Trypsin catalyses the hydrolysis of proteins into peptides and provides substrates for further enzymatic hydrolysis for protein absorption.

Pepsin, a primary protease of digestion, is also responsible for the metabolism of dietary protein.

Pepsin’s cleavage of peptide bonds is responsible for the availability of the aromatic amino acids phenylalanine, tyrosine and tryptophan. It is also responsible for the cleavage and release of several other amino acids, including valine, glycine, histamine, glutamine, alanine and leucine.

Lipase participates in cell signaling, inflammation and metabolism. Pancreatic lipase is the catalyst for the hydrolysis of dietary lipids, which include fats, oils, cholesterol esters and triglycerides [94]. Triglyceride triester is metabolized for utilization as glucose and three fatty acids. Glyphosate integration into and inhibition of lipase could induce excessive bioaccumulation of fatty material in the blood vessels, gut, liver, spleen and other organs, as well as mimic lysosomal acid lipase deficiency. It would also allow for an increase in triglycerides in the blood, leading to numerous disease cascades, including malabsorption, fatty liver disease, jaundice, failure to thrive in infants, calcification of the adrenal gland, anaemia, hypercholesterolaemia, biliary dysfunction, decreased HDL, increased LDL, blood clots, fat-enlarged hepatocytes and liver fibrosis and failure. Samsel found that radiolabeled glyphosate was not detectable by HPLC–MSMS in samples of lipase deliberately spiked for analysis, suggesting that glyphosate

may irreversibly inhibit lipase. On the other hand, pepsin and trypsin had good spike recoveries, demonstrating reversibility as glyphosate was released from the protein.

Table 3. Integration of glyphosate residues in various proteins, assessed using three testing methods.[a]

Protein substrate (Method) Bone (ELISA)

Bone marrow (ELISA) Bone (ELISA) Skin (ELISA)

Gelatin (ELISA)

Collagen (ELISA) Collagen (GC-MS) Collagen (HPLC-MSMS)

Pepsin (ELISA) Pepsin (GC-MS) Pepsin (HPLC-MSMS) Trypsin (ELISA) Lipase (ELISA)

Bee bread (HPLC-MSMS) Bees (HPLC-MSMS) Honey & comb (HPLC-MSMS)

Type Bovine leg

Bovine leg marrow Porcine foot Porcine 0.325

Bovine, Sigma Aldrich, gel strength 225 Type B

Bovine I & III Bovine I & III Bovine I & III

Purified porcine enzyme Purified porcine enzyme Purified porcine enzyme Purified porcine enzyme Purified porcine enzyme

Bee bread

Apis mellifera

Honey

2.04

120.18 130 μg/kg 95 μg/kg

< 40.00 430 μg/kg 290 μg/kg 61.99 24.43

2300 μg/kg < 10 μg/kg trace < 10 μg/kg trace

Glyphosate residue (ppb) 11.56

4.22 9.81

[a] The trace amount found in the bee substrates appeared as a small peak, which directly corresponded to glyphosate, complete with retention time and molecular features confirming contamination using HPLC–MSMS.

Table 3 shows results for various bovine and porcine products, including enzymes, bone, bone marrow, skin, collagen and gelatin. Acid hydrolysis was used on the bovine and porcine skin, bones and marrow, which were shaken and digested with 0.15 M hydrochloric acid for 24 h. The analysis methods were ELISA, gas chroma- tography–mass spectrometry (GC–MS) and HPLC– MSMS. All of the tested products were contaminated, with the highest level detected being 430 μg/kg in porcine pepsin (via GC–MS).

Additional evidence of glyphosate accumulation was found by Samsel in 2015 in the bodies of dead bees, bee bread and honey from bee hives suspected of colony collapse disorder (CCD), and these are also shown in the table. Colony collapse disorder (CCD) is an ever- increasing problem threatening pollination of crops globally. It may share a similar aetiology to that of Alzheimer's disease with regard to learning and memory within the bee's brain. Integration of glyphosate with the structural proteins and enzymes of the bee may affect protein folding and function. Additionally, glyphosate may also affect the digestive enzymes and bacterial homeostasis within the digestive system, which in turn may affect the quality of the honey produced. Glyphosate in bees may become part of their chitin, which has a structural function, in their bodies, analogous to glyphosate becoming part of the collagens of humans and other animals.

The results in Table 3 show ubiquitous contamination of the bee and bee products. Honey is derived from nectar and is the source of carbohydrates in the bee diet, whereas pollen turned into bee bread supplies the fats and proteins. Royal jelly, made from the secretions of the glands found in the hypopharynx of the worker bees, is fed to the queen and developing larvae [96].

Results for nineteen different vaccines, from five manufacturers, are shown in

Table 4. Some vaccines do not contain live viruses and do not involve gelatin in their preparation, but many involve the use of eggs, bovine calf serum, fetal bovine serum or bovine proteins [95]. Engerix Hepatitis B vaccine is manufactured through a novel procedure, which involves culturing genetically engineered *Saccharomyces cerevisiae* yeast cells that carry the surface antigen gene of the hepatitis B virus. The procedures result in a product that can contain up to 5% yeast proteins, which could be a source of glyphosate if the yeast is grown on broths or media that utilize glyphosate-contaminated nutrient sources such as animal or plant proteins.

Vaccines that tested negative for glyphosate included Merck's Hep-B vaccine, most of the pneumococcal vaccines and the sterile diluent included as a control. Gelatin is not listed as an ingredient in any of these vaccines, nor is bovine serum. In contrast, all of the vaccines that listed gelatin as an excipient tested positive for glyphosate, and nearly all of them also included bovine serum (including Varicella, MMR-II, MMRV and Zoster).

It is significant that MMR-II consistently contained the highest levels of glyphosate, significantly more than any of the other vaccines. This vaccine uses up to 12% hydrolysed gelatin as an excipient–stabilizer; as well as foetal bovine serum albumin, human serum albumin and residual chick embryo; all of which are contaminated by glyphosate during animal production.

7. EVIDENCEFORAROLEFORCOLLAGENINVACCINE ADVERSE REACTIONS

Post-vaccination allergic reactions to MMR and varicella vaccines have been linked to the gelatin excipient, and confirmed through observation of induced gelatin- specific IgE antibodies [97–100]. 24 out of 26 children with allergic reactions to vaccines (e.g., anaphylactic shock) had anti-gelatin IgE ranging from 1.2 to 250 ⌈g/mL. Seven were allergic to gelatin-containing foods. A pool of 26 control children all tested negative for anti-gelatin IgE [99]. A study from 2009 that looked at gelatin sensitivity in children who were sensitive to cows' milk, beef and/or pork as determined by IgE antibody levels [101] found that 16% of beef-sensitized children and 38% of pork-sensitized children had IgE antibodies to beef- or pork- derived gelatins that were cross-reactive with each other.

In a published case study, a 2-month-old baby developed Kawasaki disease one day after receiving its first dose of Infanrix (DTaP-IPV-Hib) and

Prevenar, a pneumococcal conjugate vaccine [102]. Kawasaki disease is an acute, multisystemic vasculitis whose occurrence very early in life is extremely rare. Extensive tests for the presence of infection with multiple bacteria and viruses were all negative. We suggest that glyphosate contamination in one or both of the vaccines may have contributed to the vasculitis through glyphosate uptake into common proteins such as collagen in the vasculature to induce the autoimmune reaction.

Kelso (1993) reported the case of a 17-year-old girl who experienced anaphylaxis within minutes of receiving an MMR vaccine [98]. The girl described the event as “kind of like what happens when I eat Jell-O[2]”. Further testing found gelatin to be the component of the vaccine

[2] Jell-O is a proprietary brand of gelatin-based desserts, popular in the USA, and manufactured by Kraft Foods, part of the Kraft Heinz Company, headquartered in Chicago.

Table 4. Glyphosate levels in vaccines determined by ELISA reported to the US CDC, NIH, FDA and UN WHO of the Americas in September 2016 by Samsel Environmental & Public Health Services.[a]

Vaccine undiluted

DTaP ADACEL

DTaP

DTaP ADACEL

HEPATITIS-B

HEPATITIS ENGERIX-B INFLUENZA FLUZONE QUAD INFLUENZA

Pneumococcal PNEUMOVAX 23 MMR II

MMR II MMR II MMR II MMRV PROQUAD MMRV PROQUAD MRV PROQUAD

Pneumococcal PNEUMOVAX 23 Pneumococcal PREVNAR 13 Pneumococcal PNEUMOVAX 23 STERILE DILUENT

VARI CELLA VARI VAX MVARICELLA VARI VAX ZOSTER ZOSTAVAX ZOSTER ZOSTAVAX ZOSTER ZOSTAVAX

Manufacturer

SANOFI PASTEUR NDC SANOFI PASTEUR

SANOFI PASTEUR MERCK

GLAXOSMI TH - KLINE SANOFI PASTEUR

NOVARTIS

MERCK

MERCK

MERCK

MERCK

MERCK

MERCK

MERCK

MERCK

MERCK

WYETH

MERCK

MERCK, SHARP & DOHME MERCK

MERCK MERCK MERCK MERCK

Lot number Exp date

58160-820-43 3-30-2018 C50418A 9-2-2018 NDC 58160-820-43 3-30-2018 LO16427 4-13-2017

NDC 58160-820-43 6- 1-20 18 6762 6- 30-2 01 6

1573 3P 05/2016 700281601 5- 18-2 01 7 7002151400 9-9-2017 009545 3-19-2017 7002151400 9-9-2017 7002151400 9-9-2017 7002305700 9-12-2017 7002305700 9-12-2017 7002305700 9-12-2017 700281601 5- 18-2 01 7 73332 07/2017 7002681601 5- 18-2 01 7 LO40058

5-11-2018 7002025000 2- 8-20 18 7002025000 2- 8-20 18 7002502401 6- 1-20 17 7002602401 6- 1-20 17 7002602401 6- 1-20 17

Test date Lab #

7-15-2016 LAB #1 5-11-2016 LAB #1 7-12-2016 LAB #2 5-11-2016 LAB #1 7-15-2016 LAB #1 7-15-2016 LAB #1 5-11-2016 LAB #1 9-19-2016 LAB #1 7-15-2016 LAB #1 5-11-2016 LAB #1 9-19-2016 LAB #1 7-12-2016 LAB #2 9-19-2016 LAB #1 7-15–2016 LAB #1 7-12-2016 LAB #2 7-15-2016 LAB #1

5-11-2016 LAB #1 7-12-2016 LAB #2 7-15-2016 LAB #1 7-15–2016 LAB #1 7-12-2016 LAB #2 9-19-2016 LAB #1 7-15-2016 LAB #1 7-12-2016 LAB #2

Glyphosate residue (ppb)

0.109

< 0.075 ND < 0.075 0.337 0.170 0.227 0.112 3.740 2.963 3.154 2.90 0.659 0.512 0.43 < 0.075 < 0.075 ND < 0.075 0.556 0.41 0.620 0.558 0.42

% Recovery in spiked sample

82%

81% - 97% 73% 95% 106% 118% - - - - 103% 86% - 77% 82% - 97% 84% - 95% 98% -

[a] Limits of detection for glyphosate in vaccines in parts per billion (ppb):[1] 0.075 (LAB #1); 0.15 (LAB #2).

to which the girl was allergic. The connexion may be to misfolded proteins, which include the collagens and associated partially hydrolysed gelatins. Indeed, both Jell-O and vaccines have been contaminated by glyphosate, as we reported in the previous section.

Puppies immunized with the rabies vaccine and a multivalent canine vaccine were compared to unvaccinated

control puppies [103]. The vaccinated puppies, but not the unvaccinated ones, developed autoantibodies to their own collagen. A follow-up study where either just the rabies vaccine or just the multivalent vaccine was administered produced a similar result. The authors suggested that this could explain issues of joint pain that are currently common among dogs, particularly as they age.

8. MULTIPLE SCLEROSIS (MS)

8.1 Sugar beet and MS

The world obtains 30% of its sugar supply from beet sugar. While sugar cane is grown in tropical regions, sugar beet requires a temperate climate. The highest incidences of MS worldwide are in the USA, Canada and western Europe [5], where most of the beet sugar is produced. MS rates are higher in the northern states of the USA compared to the south, corresponding to the distribution of sugar beet cultivation. MS rates in Canada are highest in the Alberta prairie region, at the centre of the Canadian sugar beet industry [104]. Studies on migrants have shown that those who move from a low- risk to a high-risk area tend to adopt high-risk only if they migrated during childhood [105]. This implicates local environmental factors acting before adolescence. Tokachi province in Japan hosts only 0.3% of the population, but produces 45% of the sugar beet consumed in Japan [37]; this province has the highest rate of MS among all Asian populations [106].

A fascinating proposition how sugar beet could cause MS implicates a unique noncoding amino acid that is produced by sugar beet, namely Aze. Both proline and Aze have a unique structure for an amino acid: the side chain loops back round to connect up to the nitrogen atom. In the case of Aze, there are only 3 carbons in the ring instead of the 4 carbons in proline (Fig. 2). It has been shown experimentally that Aze can be inserted by mistake into proteins in place of proline [38].

Myelin basic protein (MBP) is an essential protein for maintaining the myelin sheath, and it interacts with actin, tubulin, calmodulin and SH3 domains [107]. It

assembles actin filaments and microtubules, binds actin filaments and SH3 domains to membrane surfaces, and participates in signal transduction in oligodendrocytes and myelin. A central proline-rich region in MBP is functionally significant [108–110] and, in particular, is a binding site for Fyn-SH3, a key regulatory protein [111]. Proline substitutions of the SH3

ligand decrease its affinity for the Fyn-SH3 domain [108]. Fyn is localized to the cytoplasmic leaflet of the oligodendrocyte plasma membrane, where it participates in numerous signaling pathways during development of the central nervous system [112, 113]. Phosphorylation at a polyproline structure in the Fyn-binding region of MBP affects its structure.

A study using recombinant murine MBP inserted into *E. coli* strains demonstrated conclusively that Aze makes its way into MBP, substituting for up to three of the eleven possible proline sites. Molecular modeling of a proline-rich region of the recombinant MBP illustrated that misincorporation of Aze at any site would cause a severe bend in the polypeptide chain, and that multiple Aze substitutions would completely disrupt the structure of MBP [114, 115].

A possible concern regarding Aze is that over 90% of the sugar beet grown in the USA and Canada is genetically engineered to resist glyphosate. Therefore, the crops are exposed to significant amounts of glyphosate. The electronic *Code of Federal Regulations e-CFR 180.364 Glyphosate; Tolerances for Residues*, allows up to 25 ppm residue of glyphosate in dried sugar beet pulp. In 1999, Monsanto realized that its GM sugar beet crop well exceeded the upper limit established by the EPA for glyphosate residues. They requested, and were granted, a 125-fold increase in the upper residue limit for dried beet pulp (from 0.2 to 25 ppm). At the same time, the upper limit for fresh beet was increased fiftyfold to 10 ppm.

Glyphosate has been shown to increase the risk of root rot in sugar beet, caused by fungi [116]. Aze has been demonstrated to have antifungal activity [117]. Plants tend to increase synthesis of toxins under stress conditions, and it is plausible that an increased potential for root rot would result in increased synthesis of Aze. This is especially likely given that plants increase proline synthesis under a variety of different stress conditions [118]. However, to our knowledge, whether glyphosate causes an increase in either proline or Aze synthesis in sugar beet has not been investigated.

Consumption of milk worldwide is strongly correlated with MS risk (Spearman’s correlation test = 0.836; $P < 0.001$) [119]. For the past several decades, cows’ feed has been supplemented with either beet

Figure 2. Molecular structures of the coding amino acids proline, L-arginine, glycine and

glutamic acid; and their respective noncoding analogues Aze, L-canavanine, glyphosate and glufosinate.

molasses or sugar beet pulp, left as a residue after the sugar has been extracted [120]. Aze has been experimentally found in three sugar beet by-products that are fed to farm animals: sugar beet molasses, and both shredded and pelleted sugar beet pulp [38]. Casein is relatively enriched in proline [121]. If cows are exposed to Aze from the sugar beet, it will likely get inserted by mistake into casein, causing it to resist proteolysis. MBP's critical proline-rich sequence is vulnerable to misincorporation of Aze. The characteristic plaques of MS show loss of MBP within lesions in axon sheaths [107]. It is unclear whether this autoimmune reaction would arise through molecular mimicry from antibodies to unmetabolized peptides from casein or as a direct result of improperly folded MBP due to Aze insertion.

Glyphosate, an analogue of glycine, can be expected to be found in all tissues, including the milk of all mammals consuming glyphosate residues in the diet. Radiolabeled glyphosate studies conducted with lactating goats found ^{13}C and ^{14}C residues of glyphosate (N-phosphono- methylglycine), N-acetylglyphosate and other radiola- beled metabolites in milk. Monsanto found daily average ^{14}C residue levels from 19 to 86 ppb, with levels falling after five days of depuration to 6 ppb prior to sacrifice for organ examination. Results disseminated by Monsanto indicate that lactating animals (goats) fed a diet containing glyphosate and AMPA can be expected to have measured residue levels in edible tissues and milk [122]. In 2007 Dupont, in a similar study, examined the metabolism of N-acetylglyphosate in lactating goats. Detectable residues of N-acetylglyphosate, glyphosate and AMPA were detected in milk and other tissues. Milk, liver and kidney each contained 0.03% of the administered dose. Individual daily radiolabeled residues in the milk ranged from 0.030 to 0.036 ⌈g/g [123].

Lactobacillus plays an important rôle in metabolizing casein in the human gut. A detailed study of the prolyl aminopeptidase from *Lactobacillus*

revealed that it is a member of the class of ⟨/® hydrolases. Multiple sequence alignment has revealed three distinct highly conserved regions in this family and all three contain at least two highly conserved glycines [124] that would be vulnerable to displacement by glyphosate. The motif gly-x-ser-x-gly-gly characterizes the domain sur- rounding the catalytic serine residue of prolyl oligopeptidases in general. The glycine residues in this motif contribute to the correct positioning of the catalytic serine with respect to its substrate. A second glycine-rich domain appears essential to activity, as it likely corresponds to the oxyanion hole. The function of the third highly conserved glycine-rich domain, with the motif asp-x-x-gly-x-gly-x-ser, remains unknown. *Lactobacillus*

spp. are also highly dependent on manganese to protect them from oxidative damage, hence glyphosate's preferential chelation of manganese likely harms *Lactobacillus* [125].

An examination of collagen in the jugular veins of MS patients undergoing surgical reconstruction revealed an abnormal collagen structure, characterized by thin, loosely packed type III fibres [126]. Collagen is rich in proline. If too many of the prolines in procollagen are displaced by Aze, the polypeptide does not fold into a stable triple-helical conformation, which is a prerequisite for normal secretion of procollagen [127]. This reduces the release of procollagen and the misfolded molecules are subjected to proteolysis for recycling. resulting in the useless expenditure of energy for building and degrading procollagen molecules. Those that are released can be expected to produce defective collagen matrices. Collagen is even more highly enriched in glycine than in proline, as its core structure consists of a triple peptide repeat, where glycine is always the third residue of the triplet, and proline and hydroxproline often occupy the other two positions [128]. Glyphosate substitution for glycine in structural proteins; i.e., collagen, elastin, fibronectin and laminin; would contribute to disrupted folding as well as defective strength and elasticity.

Conserved prolines also play a crucial rôle in ion channel gating, the regulation of hypoxia-inducible factor (HIF) and embroygenesis; in fact, substituting Aze for proline is a technique used to test whether a particular proline residue is critical to the protein's proper functioning [37].

8.2 Rôle of *Acinetobacter* and *Pseudomonas aeruginosa* in MS

A series of papers by Ebringer et al. have suggested an important rôle for the Gram-negative bacteria *Acinetobacter* and *Pseudomonas aeruginosa* in MS [129–131] as well as a proposed link to prion diseases. Their most recent paper in *Medical Hypotheses* presents the evidence to support this idea from multiple dimensions [130]. First, MS patients were shown to have elevated levels of antibodies to these two microbes but not to the common gut microbe *E. coli* [132, 116]. They have autoantibodies to MBP and myelin oligodendrocyte glycoprotein (MOG) [131]. MS patients are also prone to sinusitis and *Acinetobacter* is one of the most common microbes found in nasal sinuses. Ebringer et al. also proposed that the increased prevalence of sinusitis in colder climates may explain the geographical distribution of MS in more northerly latitudes [130]. *P. aeruginosa* causes upper respiratory infections and it is among the microbes that have developed multiple antibiotic

resistance in recent years, presenting a huge problem in hospital infection [133]. *Acinetobacter* has also become resistant to multiple antibiotics [134].

The number of microbial species that can metabolize glyphosate is quite small. A 1996 study showed that *Acinetobacter* is able to fully metabolize both glyphosate and AMPA and utilize these molecules as a source of phosphorus [135]. A study of agricultural soil heavily polluted with glyphosate identified only three species capable of degrading glyphosate when exposed at a level of 1000 ppm: *Pseudomonas putida*, *P. aeruginosa* and *Acetobacter faecalis* [136]. Another study on marine species identified *Pseudomonas* as being among the rare microbial species that can utilize the phosphonate in glyphosate as a source of phosphorus [137]. It can be predicted that *Pseudomonas* and *Acinetobacter* species in the nasal or digestive tracts would have a substantial advantage over other microbes if they can degrade glyphosate. On the other hand, they would also be heavily exposed if they actively take it up, and it would not be unreasonable to

assume that some of the glyphosate might end up in their synthesized proteins by mistake in place of glycine. Both *Pseudomonas aeruginosa* and *Acinetobacter* strains have recently become a serious problem in hospitals, and a public health issue, due to their multiple-antibiotic resistance [138]. Glyphosate has been

shown to induce generic antibiotic resistance in other microbial species, including *E. coli* and *Salmonella*, through the induction of a generic capability to export toxic chemicals through efflux pumps [139].

A PEP transferase enzyme synthesized by *Acinetobacter calcaceticus* has sequence homology with a bovine prion sequence, and antibodies against synthetic peptides containing the structurally related sequences were found to be significantly elevated in cattle with bovine spongiform encephalopathy (BSE) compared to negative controls [140]. Ebringer et al. (2005) [129] link MS to BSE, also known as “mad cow disease”, and to the related human disease, Creutzfeldt– Jakob disease (CJD). Cows suffering from BSE manifest hindquarters paralysis early after onset, similar to the mobility issues afflicting MS patients at onset. Ebringer et al. found elevated levels of antibodies to both *Acinetobacter* and *Pseudomonas*, along with autoantibodies to both white and grey matter components, in BSE-affected animals, as is also the case for MS [129].

Of particular note are the molecular similarities they identified between certain peptides found in these two microbes and peptides in MOG and MBP that are known to be allergenic. Strikingly, all three of the microbial sequences they identified and all three of their human protein analogues contain conserved glycines (Table 5).

Table 5. Amino acid sequences of three peptides from *Acinetobacter* and *Pseudomonas* and the corresponding human peptides from MBP that they mimic.[a]

Microbe

Protein Peptide MBP

Acinetobacter 3-OACT-A Leu-Tyr-Arg-Ala-Gly-Lys Leu-Tyr-Arg-Asp-Gly-Lys

Acinetobacter 4-CMLD Ser-Arg-Phe-Ala-Tyr-Gly Ser-Arg-Phe-Ser-Tyr-Gly

Pseudomonas

Gamma-CMLD T h r - A r g - H i s - A l a - T y r - G l y S er - A r g - P h e - S e r - T y r - G l y

[a] Note that all six peptides have a glycine residue.

MOG is strongly implicated in the disease pathology of MS; autoantibodies recognizing MOG have been found in the CNS of MS patients [141]. One of the major encephalitogenic peptides in MOG is the sequence from residue 92 to residue 106, which contains a highly conserved glycine near its centre [142].

Both diabetes and MS are associated with abnormal T- cell immunity to proteins found in cow's milk [143]. In a study conducted in dairy cows by Monsanto in 1973, ^{14}C- radiolabeled glyphosate was studied in the distribution of residues in milk, urine, faeces and other tissues of the lactating cow. Glyphosate contamination of milk ranged from 9 to 15 ppb with the highest accumulation in the kidney and rumen fluid (201 ppb and 109 ppb, respectively) [201]. An epitope of bovine serine albumin found in milk that is linked to MS but not to diabetes is BSA193. It shows

structural homology with exon 2 of MBP through the peptide sequence GLCHMYK. Note that the first peptide in this sequence is glycine. Exon 2 is a target peptide in both MS autoimmunity and in experimental autoimmune encephalitis (EAE), an animal model of MS [144–146]. Exon 2 of MBP is implicated in remyelination [144]. Its expression is largely restricted to the developing brain and to areas of myelin reconstruction, notably MS lesions [147].

The gly-ser-gly-lys tetrapeptide is highly conserved among MBPs from multiple species [148]. The serine in this sequence is the site of attachment of polyphosphoinositide. The highly conserved nature of this sequence suggests that the phospholipidation of MBP is important biologically. Substitution of glyphosate for either of the glycines would likely disrupt this modification.

9. MMRVACCINEANDAUTISM

In this section, we make a case for a direct link between the measles, mumps, and rubella (MMR) vaccine and autism, via autoantibody induction through molecular mimicry. In a paper provocatively titled, “Peptide cross- reactivity: the original sin of vaccines”, Kanduc makes the point that massive cross-reactivity between antigens in vaccines and similar sequences in human proteins makes it almost inevitable that vaccines lead to autoimmune disease through molecular mimicry [149]. Reported post-vaccination autoimmune diseases include systemic lupus erythematosus, rheumatoid arthritis, inflammatory myopathies, multiple sclerosis, Guillain– Barré syndrome and vasculitis [150].

It is becoming increasingly acknowledged that autism may be an autoimmune disease. Family members of autistic children have a significant increased risk to other known autoimmune diseases such as hypothyroidism, rheumatic fever and multiple sclerosis [151]. Several studies on both humans and monkeys have revealed a potential link between maternal antibodies directed against specific foetal brain proteins and a future autism diagnosis in the foetus [152–155]. Furthermore, it has already been demonstrated that vaccines are capable of inducing autoimmune antibodies against proteins in the brain. The narcolepsy epidemic in Europe following an aggressive immunization campaign against the H1N1 ’flu virus was eventually conclusively resolved as being attributed to autoimmune reactions to the hypocretin receptor through molecular mimicry from a peptide in the surface-exposed region of the influenza nucleoprotein A that was present in the H1N1 vaccine [156] (hypocretin is an important regulator of sleep).

Much controversy surrounds the concept that the MMR vaccine may be contributing to the autism epidemic in the USA and elsewhere. In an immune- compromised child, the live measles virus from the vaccine is capable of infecting the brain and sustaining a chronic measles infection, resulting in loss of neurons, eosinophilic intranuclear inclusions and gliosis, a condition termed “subacute measles encephalitis”. This can result in a seizure disorder and developmental delay in language and motor skills (as was clearly observed in a case study involving an HIV-positive 2-year-old boy [157]).

Singh et al. have published a series of papers over the past two decades [14, 158–160] proposing that there is a subpopulation among the autism community who can be characterized as suffering from “autoimmune autistic disorder” [14]. The 1998 study by Singh et al. found that 90% of measles-IgG-positive autistic sera were also positive for anti-MBP antibodies, supporting the hypothesis that a virus-induced autoimmune response may be

causal in autism [158]. A follow-on serologic study of antibodies to viruses associated with autism published in 2003 revealed a statistically significantly elevated level of measles antibody in children with autism compared to their siblings ($P = 0.0001$) or to unrelated children ($P = 0.003$), but not with antibodies to mumps or rubella [159]. In a later study, 60% of 125 autistic children had significantly elevated levels of antibodies to measles haemagglutinin unique to the MMR strain of the virus, compared to the 92 control children [160]. Over 90% of the children who had elevated antibody levels also tested positive for MBP autoantibodies. It was suggested that this could be linked to virus-induced autoimmunity through mimicry.

In fact, there is a sequence homology of 78% between a peptide sequence from MBP (EISFKLGQEGRDSRSGTP) and one found in a measles virus protein, MP3 (EISDNLGQEGRASTSGTP) [161, Table 2, p. 7]. Three of the matches between these two sequences are glycines. Measles virus-neutralizing antibodies are mainly directed to haemagglutinin, implying that it is essential for acquired immunity from the vaccine [162]; yet over-production, particularly if the virus penetrates the blood–brain barrier, runs the risk of inducing an autoimmune response to the myelin sheath. In fact, high measles antibody titres have been previously linked to MS [163].

Gonzalez-Granow et al. found high titres of autoanti- bodies in both the IgG and IgA classes specific to MBP in the serum of patients with autism [15]. The IgA antibodies in particular were shown to act as serine proteinases to degrade MBP *in vitro*. They also induced a decrease in long-term potentiation in perfused rat hippocampi. Reduced long-term potentiation in the hippocampus is a feature of autism, as has been clearly demonstrated in studies using mouse models of autism [164].

Dr Andrew Wakefield was the first to reveal a possible connexion between

MMR and autism. His controversial *Lancet* paper, published in 1998 and then later retracted, proposed that this vaccine caused an acute reaction in children with gut dysbiosis (abdominal pain, diarrhoea, food intolerances, bloating etc.) [9]. The paper reported on a group of 12 children who had experienced developmental delay following an MMR vaccine and who were diagnosed with autism. These children suffered from rash, fever, delirium and seizures following the vaccination with MMR. He and several colleagues later published additional papers elaborating the hypothesis that dysbiosis in the gut, combined with impaired protein hydrolysis, leads to autoimmune lesions in the duodenum that are associated with extensive colonic lymphoid hyperplasia. The release of undigested peptides

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into the vasculature across a leaky gut barrier and, ultimately, from the vasculature across a leaky blood–brain barrier, could induce encephalopathy [165–167].

In an epidemiological study from 1998, encepha- lopathy was clearly demonstrated as an acute reaction to measles vaccine, where 48 cases were found following vaccination, with no cases identified after administration of either monovalent mumps or rubella [168]. Among these 48 children, eight died, and the remainder experienced mental regression, chronic seizures, movement disorders and sensory deficits in the subsequent months.

The FDA's vaccine adverse event reporting system (VAERS) database is a valuable tool for uncovering trends in vaccine adverse reactions. Our earlier studies on VAERS comparing MMR with an age-matched, equal- sized distribution of all other vaccines showed a significant association of MMR with autism ($P < 0.007$) [169]. This was puzzling, because MMR has never contained either aluminium or mercury, the two prime candidates for the kind of neurological damage that might lead to autism [170–174]. Strong associations also appeared with fever and rash. In that paper, we proposed that the adverse reaction might be caused by the acetaminophen administered to the child to try to curb the seizures.

Since glyphosate usage on crops has gone up dramatically since the GM Roundup Ready crops were

first introduced in 1996, we decided it would be worthwhile to compare the early data on MMR in VAERS with the later data. We defined a cutoff date on 1 January 2003, such that the events where MMR was included as an administered vaccine could be separated into “early” and “late”, based on whether they were before or after that date. Each dataset represented a 13-year interval. We found 10 639 events in the early set and 19 447 events in the late set; thus, the raw number of events nearly doubled in the later years.

We also tabulated the frequency of different adverse reactions in the two sets, and used a standard statistical analysis to compute the significance of any differences observed: we randomly down-sampled both sets as needed such that there was an identical total count and an identical distribution over age in the two datasets. Results were surprising: many symptoms associated with atopy or with an allergic reaction were significantly higher in the later set, and “hospitalization” was highly significantly overrepresented in the later set [Table 6]. Other overrepresented symptoms included seizures, dyspnea, hyperventilation, asthma, eczema, autism, hives, anaphylatic [shock], and irregular heart rate. Interestingly, the early set had more frequent occurrences of joint pain and arthritis, suggesting that the toxic elements in the vaccine impacted the joints rather than the brain.

Table 6. Frequency of various adverse reactions to MMR before and after January 2003 [US FDA, VAERS]. The *P*-values were computed according to a χ^2 goodness-of-fit test.

More common before 2003

Reaction

Art hri ti s Joint pain

Reaction

Hospital Seizures Dyspnea Hives Anaphylactic Eczema Autism

Hyp er ve nt il at io n General infection Asthma Immunoglobulin G Ear infection Heart rate irregular

Count < 2003

5 2 175

Count ≥ 2003

18 75

P-value

0. 045 0.012

P-value

0.00041 0.0055 0.0086 0. 011 0. 017 0. 028 0. 031 0. 035 0. 044 0. 046 0. 048 0. 048 0. 049

More common after 2002

Count < 2003

132 314 139 444 28 10 105 18 77 22 0 32 11

Count ≥ 2003 423 534 279 654 91 47 184 57 136 58 17 72 39

To our knowledge, there have been no significant changes to the formulation of MMR since its introduction. The explanation for the significant changes in adverse reactions must, therefore, lie in external factors, one of which is likely to be glyphosate. We suggest that both chronic exposure to glyphosate from food, water and air and direct exposure to glyphosate residues in the vaccine are relevant factors. A child with a disrupted gut microbiome due to chronic glyphosate exposure will also suffer from a leaky blood–brain barrier, and this will lead to a much greater possibility of measles antigenic proteins entering the brain and causing anaphylaxis and seizures.

The measles virus is a member of the family of paramyxoviruses, which have two highly-conserved glycine residues at positions 3 and 7 in the hydrophobic fusion peptide (FP) region of the viral fusion-mediating glycoproteins [175]. This FP region is the most highly conserved region of the glycoproteins, and it plays a critical rôle in destabilizing the membrane of the host cell to gain entry. Substitutions of other amino acids for either the

G3A or G7A glycines caused increases in both cell– cell fusion and the reactivity of the protein to antibodies, leading to both a higher infection rate and increased chances for an autoimmune reaction. Glyphosate substitution is likely to do the same, as well as leading to a form of the protein that would resist proteolysis.

The FPs of both the influenza virus and human immunodeficiency virus (HIV) gp41 contain numerous glycine residues at regular intervals, with glycine overall making up 29 and 26%, respectively, of the total peptide sequence [175]. Optic neuritis, an immune-mediated demyelinating injury of the optic nerve, has been recognized as a side effect of the influenza vaccine that can lead to blindness [176].

10. OTHERAUTOIMMUNEDISEASES

10.1 Neuromyelitis optica and aquiporin

Neuromyelitis optica is a rare severe inflammatory demyelinating disorder of the central nervous system, which is related to multiple sclerosis but distinctly different and manifested mainly by paralysis and optic nerve damage [177, 178]. It has been conclusively demonstrated that this condition is caused by an autoimmune reaction to aquaporin-4, which is highly expressed in the astrocyte membrane [177, 178].

Aquaporins are important membrane proteins, which can transport water molecules through pores into the cell while excluding protons [179]. They are highly expressed by astrocytes, one of whose rôles is to mediate water flow among the vasculature, the

cerebrospinal fluid and the lymph system [178]. Thus, aquaporins are implicated in brain oedema [180]. Plants produce aquaporins as well, and mimicry between plant and human aquaporins has been proposed as a mechanism for the development of an autoimmune sensitivity to this protein [181]. Plants considered to show aquaporin mimicry notably include corn and soy as well as tomato, tobacco and spinach [182].

Autoimmune sensitivity to aquaporin has also been found in association with MS [182]. Vojdani et al. found significant elevations in antibodies against both human and plant aquaporin 4, in addition to antibodies against MB,

MOG and S100 calcium-binding protein B (S100B) in patients suffering from MS.

Among the aquaporins, aquaporin-6 is unique in that it operates as an anion channel instead of as a water channel. Analysis of the peptide sequence in comparison to other aquaporins reveals that aquaporin-6 has an asparagine substituted in place of a glycine at residue 60. This one small difference completely changes the way the molecule behaves in the membrane. A glycine at this position is conserved among all the other aquaporins. Furthermore, aquaporins are constructed of ⟨-helices, and there are three sites where the helices cross. Highly conserved glycine residues are found at all three sites [57, 183].

Aquaporin is also found in bacteria, although homology with human aquaporin is only about 20%. The bacterial aquaporin is a 27 kDa trypsin-resistant protein called aquaporin-Z, which was originally described in *E. coli* [184]. Sequence analysis conducted by Ren et al. [185] revealed four regions where homology was considerably stronger (90%, 60%, 50% and 45% respectively). They convincingly showed cross immunore- activity between the human and bacterial versions of the protein. Antibodies to aquaporin Z bind to astrocytes, activate complement, and cause death.

Ren et al. [185] identified all the residues where the bacterial and human peptides were identical (Fig. 1 in [185]). A tally of counts reveals that glycine was by far the most common among these matched residues, representing 14 of the total 66 matches. The second most common amino acid was lysine with 8 matches. Alanine, isoleucine and valine had 7, 5 and 4 matches respectively, and all other amino acids had less than four.

Thus, it appears that glyphosate-substituted trypsin- resistant aquaporin from both gut microbes and from GM glyphosate-resistant corn and soy foods are plausible sources of antigens that could induce neuromyelitis optica and contribute to the disease process in MS through misincorporation.

10.2 Type 1 diabetes

Type 1 diabetes is considered a genetic disease, but its incidence has been increasing by 3–4% worldwide every year in the recent past [186, 168]. Although an environ- mental component is highly suspected, environmental factors have not yet been identified. An increased incidence of type 1 diabetes is associated with both MS [187] and autism [188]. The disease is characterized by an autoimmune reaction to various proteins expressed in the pancreatic islet cells. Specifically, antibodies against glutamic acid decarboxylase (GAD65) are often found [189]. Cross-reactivity with proteins from foods and microbes in the gut are both possibilities.

One microbe that may be inducing antibody production through mimicry is *Mycobacterium avium paratuberculosis* (MAP). Blast analysis revealed 75% homology between a previously identified antigenic region of GAD65 [190] and a MAP heat-shock protein (HSP65) [189]. The specific 16-residue matched sequence in HSP65 centrally contains a pair of glycines which could be substituted by glyphosate to cause resistance to proteolysis. This microbe has been linked to numerous other human diseases including ulcerative colitis, irritable bowel syndrome, sarcoidosis, Hashimoto's thyroiditis, MS and autism [188]. With respect to MS and autism, cross- reactivity between HSP65 and MBP through mimicry may provide the link.

Patients with type-1 diabetes commonly have an antibody reaction to bovine serum albumin, a component of cows' milk [191]. The hypothesized explanation is an autoimmune reaction to a beta-cell specific surface protein through mimicry.

Insulin-derived amyloidosis is a condition that can develop following long-term insulin therapy, whereby an "insulin ball" develops at the site of injection. This hard mass has been analysed and found to contain accumulations of insulin fibrils reminiscent of amyloid ®-plaque in the Alzheimer's brain. Insulin amyloidosis is more common for animal (cows and pigs)-derived than human-derived insulin products. Nowadays, cows and pigs are chronically exposed to glyphosate in their feed. The rôle of glycine residues in proteins may indeed be to protect from aggregation into amyloid fibrils [192]. Substitution of glyphosate for any of these conserved glycines would therefore tend to promote amyloidosis.

Glutamic acid and glycine are by far the largest component amino acids of bovine proinsulin and make up 25% of the amino acid residues in the molecule [193]. The same is true for human insulin, which differs very little from the animal versions. The herbicide glufosinate is a natural noncoding amino acid analogue of glutamic

acid (Fig. 2). Substitution of either glufosinate for glutamic acid or glyphosate for glycine in insulin is likely to impair its function, and may also lead to amyloidosis.

The widespread appearance of glyphosate-resistant weeds among the glyphosate-resistant crops has forced some farmers to turn to glufosinate as the herbicide of choice [194]. Glufosinate-tolerant corn and soybean have been available on the US market since their approval by the USDA in 1995 and 1996, respectively. A tri-resistant form of soybean tolerant of glyphosate, glufosinate, and 2,4-D was approved by the FDA in September 2014. Dual resistance to glufosinate and glyphosate in corn was approved in November 2015.

10.3 Coeliac disease

Coeliac disease and, more generally, gluten intolerance, have reached epidemic proportions in the USA in the past decade [195]. Wheat grown there is being routinely sprayed with glyphosate for staging and desiccation just before harvest. This practice clears the field of weeds prior to harvest and planting of the next crop, but increases the amount of residual glyphosate in the grain. The practice has been increasing in popularity in step with the increase in gluten intolerance. Glyphosate is systemic in the plant and enters the seed as the plant dies, hence eventually ending up in wheat-based foods.

Proline residues make up 20% of the first 100 amino acids of both ⟨- and ©-gliadins [54]. Related proteins from rye and barley are also unusually proline-rich [56]. As we implied earlier, proline is inaccessible to most digestive proteases because the bond between the peptide nitrogen atom and the side group complicates hydrolytic attack. As a consequence, specialized prolyl aminopeptidases detach the amino-terminal proline from a peptide. These enzymes depend on manganese as a catalyst, and manganese is one of the metals most dramatically affected by glyphosate chelation [125].

Unhydrolysed gliadin peptides bind to HLA-DQ molecules (receptors on antigen-presenting cells) and trigger pathogenic T-cell responses [196]. Genetic variants of HLA-DQ are linked to both coeliac disease and type 1 diabetes [197, 198].

Analysis of the X-ray crystal structure of a human cytosolic prolyl aminopeptidase worked out in 2008 revealed that it is a dimer with a dependency on two manganese ions as the catalytic centres [199]. The full sequence of the catalytic domains of six prolyl peptidases from both human and microbial species is shown in Fig. 6 in ref. 199. Six of the twenty sites of fully conserved residues across all species were glycine residues, three were histidine, two were tyrosine and two were proline. The remaining seven were seven different amino acids.

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11. CONCLUSION

In this paper, we have shown that widespread misincorporation of glyphosate for glycine during protein synthesis could explain the aetiology of multiple autoimmune diseases that are currently increasing in incidence in the USA. Misincorporation is plausible by analogy with multiple known toxins produced by organisms in defence against pathogens, including Aze, BMAA, L-canavanine and glufosinate, which work in a similar manner. We have shown that proteins from foods such as milk, wheat and sugar beet, as well as peptides derived from microbes resident in the gut or nasal tract or introduced iatrogenically through vaccination, are all potential causes of autoimmune disease induced through molecular mimicry. It is highly significant that two microbes linked to MS through molecular mimicry are among the very few microbes that can fully metabolize glyphosate. Using the VAERS database, we have shown that severe adverse reactions to the MMR vaccine have increased significantly over the past decade in step with the increased use of glyphosate. Glyphosate in MMR may originate from growth of the live virus on culture materials derived from glyphosate-exposed animals and/ or from gelatin used as an excipient stabilizer. We have confirmed the presence of

glyphosate contamination in MMR and in many other vaccines where the live virus is cultured in eggs, bovine protein or gelatin, or where animal products are used as an excipient component. Notably, some vaccines prepared without live culture on gelatin were free of glyphosate contamination. Substitution of glyphosate for glycine during protein synthesis could yield a peptide that resists proteolysis, making it more likely to induce an immune response. Furthermore, enzymes involved in proteolysis are likely to be disrupted due to their confirmed contamination with glyphosate. A non-exhaustive list of possible diseases that can be attributed to this mechanism include autism, multiple sclerosis, type 1 diabetes, coeliac disease, inflammatory bowel disease and neuromyelitis optica.

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1.40 1.20 1.00 0.80 0.60 0.40 0.20 0.00

1987 1997

Figure 1. Estimated annual world glyphosate usage.

year

2007 2017

glyphosate / million tonnes

The widespread contamination of the environment with glyphosate is just one facet of the environmental degradation assessed by the UN High-level Panel [12] (although glyphosate is not explicitly referred to in their report). It is clear that degradation such as that of the Aral Sea and its basin (also not explicitly referred to in the report [12]—evidently the Panel operated on too

high a level to perceive such details) constitutes a colossal assault on health. Nevertheless, the effects are localized (albeit over a large area) and the adaptive response of simply abandoning the area would completely eliminate the deleterious effects on health (other than the psychological ones associated with being forced to leave one's homeland). Our main concern in this essay is with degradation that is endemic in the developing world; that is, alongside a high degree of material development. In fact, pollution is a corollary of such development; such as the emission of fumes from motor vehicles.[1] The ingestion of pollutants from the air may cause not only physiological but also psychological disorders [17]. Contamination of food, including water, is widespread with far-reaching implications (e.g., [16]). Ref. 16 is concerned with glyphosate; other widespread pollutants include aluminium [18] and microplastics.[2] Of particular concern are pre- and post-natal effects of pollutants (the concept of developmental origins of health and disease, DOHaD) [20]; these could result in more or less permanent (i.e., throughout adult life) adverse changes. Less directly, pollution makes outdoor life less attractive, discouraging healthy exercise.

These threats and challenges are essentially man- made. Others, including infectious pathogens not so far affected by resistance to antibiotics, are natural. A major natural geophysical (with profound implications for the biosphere) challenge is climate change.[3] Since modern man emerged some 200,000 years ago, he has had to endure many phases of cooling and warming, and his survival so far attests his adaptive capability.[4] Adaptation can take place on three distinctive timescales: proximate, behavioural adaptation is essentially instantaneous; ontogenetic adaptation takes place over the lifetime of an organism, through changes in neurological structures

(learning) and in protein expression repertoires (epigenetics); and phylogenetic adaptation involves changes in gene structure and, ultimately, speciation. Man seems to have managed very well especially through ontogenetic adaptation. This would also cover the development and use of machines and other technologies directed towards dominating nature. The current trend of worsening health suggests that our adaptive capacities are becoming limited.

Boyden [23] has argued that man in the midst of 20th- century civilization suffers from phylogenetic maladjust- ment because the conditions of life

nowadays are different from those prevailing when he emerged as a species. Differences there undoubtedly are, but even since the beginning of agriculture and pastoralism there have been several hundred generations, which should be ample for enabling a degree of phylogenetic adaptation. More convincing is his concept of pseudoadaptation, in which survival occurs under changed environmental conditions without an active adaptive response from the individual, because of what Boyden calls the mollycoddling influence of civilization. It is thus an anti-Darwinian phenomenon that makes a species highly vulnerable to destruction should that mollycoddling influence suddenly disappear.

It is striking that an overwhelming majority (about 70%) of the global burden of disease is caused by poor lifestyles, whereas mortality due to infectious diseases is falling quite dramatically [24]. Prominent among these “lifestyle diseases” is what has been called “diabesity” [25], a combination of diabetes and obesity. The official view is that diabesity is caused by a combination of overeating (especially sugary foods) and underexercising [26]. Not to be overlooked are various kinds of neuroses. We can presume that at least up to the start of the Industrial Revolution, people were generally fit, even those leading sedentary lives (think of Immanuel Kant and his daily walk). Food was much less processed and surely more wholesome than today. Mobility was generally achieved by walking. While Johann Sebastian Bach was at school in Lüneburg, he thought nothing of walking to Hamburg, about 60 km away, to listen to the organist Reinken. That all changed with the Industrial

. [1] It might be argued that corrective measures are constantly in place (such as the Alkali Inspectorate being formed to combat the hydrochloric acid emissions from soda factories). Indeed, motor vehicles are now obliged to be fitted with devices that reduce their emissions, but as the recent Volkswagen scandal demonstrated, such “obligations” are easily circumvented by the wiles of contemporary manufacturers. It is perhaps significant that one cannot imagine the engineering leaders around the time that T.H. Huxley was speaking (e.g., I.K. Brunel, who had died rather young just a few years previously) contemplating such wiliness.

. [2] Particles of synthetic polymers are now widely dispersed throughout the world’s oceans. When ingested by wildlife, including organisms used as food by humans, there may be deleterious physiological effects [19]. Unfortunately the field of microplastics research appears to have suffered breaches of integrity (see the editorial expression of concern by J. Berg published online by *Science* on 1 December 2016).

. [3] The relative weights of anthropogenic and natural factors are still being debated [21].

. [4] Adaptation is a special case of directive correlation [22].

Revolution. One simply took a train. Doubtless there are many commuters between these two places nowadays. Perhaps one's appreciation of a Reinken playing would be blunted by ears attuned to the clangour of machinery—a kind of neurosis. The machines that were used to dominate nature also came to dominate our lifestyle. The motor-car plays, perhaps, the central rôle in this domination. It is vastly overused, hence responsible for underexercising as well as air pollution and injuries. Its only redeeming merit is that it demands concentrated mental alertness from its driver, but this will vanish if autonomous cars become a reality.

The government, rather paternalistically,[5] at least in the UK, prescribes 150 minutes of exercise weekly. This is in contrast with the recommendation of a Georgian exercise manual to run or walk about 20 miles *daily* [28]—about an order of magnitude more. In those days it was not uncommon for workers to walk ten miles to their place of work, and ten miles back home. Apart from the exercise, this provided plenty of opportunity for what is nowadays called "meditation", a bulwark against neuroses. We are, of course, all still at liberty to decide how to manage our individual lifestyles. Even if we possess a motor-car it does not have to be used. Similar remarks apply to television—passively watching something instead of being active, quite apart from the dubious value of what is generally viewed [29]—which may itself contribute to neuroses [30]—and computers used for playing games, mobile devices etc.

Cellphones are considered to be very convenient and the same applies to processed foods. In the UK, the annual turnover of the food and drink manufacturing industry is about a the same as the budget of the NHS—around 110,000 million GBP. A farmer once opined to me that "the less one does to milk, the better". But, the problem is that bread does not grow in the field, nor can wine be tapped from the vineyard. As Paracelsus put it [31],

“For nature is so subtle and challenging in her things, that she cannot be exploited without great expertise. There is nothing in our world lying to hand in ready form; Man must complete it. This work of completion is called alchemy. The alchemist is like the baker who bakes bread, the vintner who makes the wine, the weaver who turns threads into cloth. Therefore whatever things are in nature which are useful to Man, these things must Man the alchemist bring to order and completion” [32]. The difficulty is to know when to stop. Something like the principle of sequential minimization of entropy loss

(SMEL) [33] may be applicable—maximizing new contacts while minimizing the loss of conformational freedom. SMEL governs the folding of biopolymers like RNA [33] and is a manifestation of the universal principle of least action. The conformational trajectory that leads to the correct final conformation is the one that minimizes the action. With food, the endpoint is less clear. Generally, one stops the processing as soon as one has a comestible product (with some subtlety: the must of grapes (siasser in Alsatian) can be drunk, but cannot be kept). This may correspond to a minimum in the minimization process. Practically speaking, the more food is processed, the less it is amenable to inspection, especially if sold in sealed packages. One can inspect grapes and have a pretty good idea of the quality of wine that could result. Fresh fruit and vegetables sold in the market, and meat from the butcher, can be carefully inspected before purchase. On the other hand the selection and quality of the ingredients in a manufactured food item are left in the hands of the manufacturer, who will presume that the consumer is a free individual, who can freely choose whether to purchase an item—as a Dutch proverb puts it, “De klant is koning” (the customer is king).[6] The consumer may indeed put a blind trust in the manufacturer, which may or may not be justified—at any rate, judging from the tenor of their advertisements, most manufacturers appear to attempt to diminish the discriminative power of would-be purchasers of their products to zero.

Processing also implies pollution and contamination. Many drinks are sold in aluminium cans, the dangers of which have already been alluded to [18]. Urbanization— which continues to increase—implies a degree of processing, if only to ensure that the food remains edible during the lengthy journey from its point of origination to its point of consumption. Hence, it is common (at least in the USA) to treat chicken meat with chlorinated water prior to

dispatch to discourage the growth of microbes. More insidiously, many manufactured drinks are now prepared with artificial sweeteners. There has long been controversy about their safety, but the most relevant aspect may not be the nature of the molecules themselves, but impurities inevitably present. Aspartame, for example, is made in a classic biotechnological fermentation process by bacteria, and is inevitably contaminated by other bacterial metabolites, some of which may have dangerous physiological consequences (such as the ability to form channels in cell membranes) (P.A. Grigoriev, personal communication).

. [5] "It is not the task of government to improve the behaviour of its 'subjects'. Neither is it the task of businessmen. They are not the guardians of their customers. If the public prefers hard to soft drinks, the entrepreneurs have to yield to these wishes..." [27].

. [6] Quoted in ref. 34.

Armed with knowledge like this, augmented by reading the scientific papers cited in the list of references, one can learn and modify one's consumption habits via ontogenic adaptation. It is surely more realistic to aim for that rather than halting urbanization, banning machines and so forth. Doing the latter implies denying man's predatory nature.

One of the complicating features is peer pressure. In general, we are not deciding what to do as an isolated individual, but are surrounded by other individuals and organizations with more or less definite ideas about what they would like to do—regardless of the quality and quantity of available evidence pertinent to their actions. There is not space now to go into this further, other than to point to the achievements of Serge Galam's sociophysics in more rationally understanding these collective effects.

It seems like a fair generalization to assert that "added value", eagerly sought after by both entrepreneurs, who see it as a source of profits, and governments, who see it as a source of taxation, is generally inimical to

health. In order to counter the adverse effects, new medicines are needed, creating further added value and contributing to GDP. Thus, for example, it is now recommended that statins are given to almost everybody [35–37] (although see refs 38 and 39 for a contrary view). That would be a good example of pseudoadaptation.

Amid the cacophony of advice offered from the Internet, by professionals, by governments, by peers and so forth, and alongside the eroding trustworthiness of scientific journals, it is understandable that a sober course of ontogenic adaptation to maintain good health might be difficult to achieve. Yet another complicating factor is venality—vested interests abound, and they may not merely be financial; some causes, such as the fluoridation of tap water, are espoused with religious fervour [40]. Only resolutely critical thinking can steer a firm and "good" (ultimately defined in terms of a focal condition [22]) course through such turbulent waters. Yet, it seems that schools, with their strong emphasis on new, Internet- based technologies, are not developing critical thinking as an aptitude [41]. Besides, if syncretism is the contemporary goal (as the politically correct would doubtless like it to be), critical thinking is not even necessary. All this augurs ill for effective ontogenetic adaptation.

A most insidious and disturbing trend is the appalling violence between human beings that seems to dominate what has rapidly become a vast genre of video games played using a computer. In developed countries, it seems that around 40% of the population play these games. During the First World War, which involved an

unprecedentedly high proportion of the civilian population getting entrained in military activity, soldiers-in-training were systematically brutalized in order to destroy our deeply rooted aversion to harming our fellow creatures; indeed, as Lorenz has pointed out [42], this was very necessary in order for the troops to be effective. Thus, brutality significantly worked itself into the population, and the trend was reinforced by the Second World War.

Lorenz has shown that this instinct of not harming fellow predators is very ancient; careful observation of wolves and the like show it to be shared by many animals [43].[7] As if the video games were not bad enough, news media strive to bring to the vast majority of the population (more than 90%) daily

televised films from theatres of war such as Syria, showing brutality in graphic detail. Such films are considered to be newsworthy and necessary to attract the viewers the broadcasters need in order to attract, in turn, advertisers (but even public stations like the BBC that are not dependent on advertisers follow the same policy). As a result, violent brutality has become omnipresent in the visual input of a significantly large proportion of the population. Clearly, destruction of the ancient instinct of solidarity—if that is indeed occurring— must be deleterious for survival of the species. It can be considered as yet another assault on health.

In conclusion, human health is indeed being subjected to a battery of assaults. Man has, however, the capacity to adapt in order to survive. If, however, the ability to adapt is impaired, future survival looks bleak.

J.J. RAMSDEN

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